Food Hydrocolloids 56 (2016) 218–226

Contents lists available at ScienceDirect

Food Hydrocolloids

journal homepage: www.elsevier.com/locate/foodhyd

Properties of partially denatured whey protein products 2: Solution flow properties

Zhuo Zhang [a], Valeria Arrighi [b], Lydia Campbell [a, c], Julien Lonchamp [a], Stephen R. Euston [a, *]

[a] Department of Food & Beverage Science, School of Life Sciences, Heriot-Watt University, Edinburgh, EH14 4AS, UK
[b] Institute of Chemical Sciences, School of Engineering and Physical Sciences, Heriot-Watt University, Edinburgh, EH14 4AS, UK
[c] Nandi Proteins Limited, Nine, Edinburgh Bioquarter, Lab 13, Edinburgh, EH16 4UX, UK

ARTICLE INFO

Article history:
Received 20 July 2015
Received in revised form
24 November 2015
Accepted 16 December 2015
Available online 22 December 2015

Keywords:
Partially denatured whey proteins
Shear rheology
Thixotropy
Non-Newtonian

ABSTRACT

Partial denaturation of whey protein concentrates has been used to make protein powders with differing viscosity properties. PDWPC particles have been manufactured to have a range of aggregate sizes (3.3 −17 μm) and structures (compact particle gel to open fibrillar gel). In solution the PDWPC samples show complex viscosity behaviour dependant on the size and morphology of the PDWPC aggregate particles. For the same protein content the compact particles have a lower viscosity than open, fibrillar particles. The viscosity also appears to depend on the surface structure of the particles, with particles of a similar size, but having a rougher surface giving higher viscosity than similar smooth particles. The viscosity of the WPC, MPWPC and PDWPC solutions are explained in terms of the postulated interactions between the protein aggregates in solution.

1. Introduction

Whey proteins are widely used as ingredients in various foods because of their nutritional quality (Harper, 2004; Madureira, Pereira, Gomes, Pintado, & Xavier Malcata, 2007; Séverina & Xia, 2005). In addition, whey proteins are also valuable functional ingredients in foods as emulsifiers, foaming agents and gelling agents. As gelling agents whey proteins are able to aggregate and form gels that improve the textural properties of food products (Kinsella & Whitehead, 1988; Lizarraga, De Piante Vicin, González, Rubiolo, & Santiago, 2006). Properties of whey protein concentrates (WPC) solutions, including their rheological behaviour, have been investigated extensively to understand the effects of factors such as protein concentration, temperature, pH and ionic strength on the molecular functionality (Hermansson, 1975; McDonough, Hargrove, Mattingly, Posati, & Alford, 1974; Pradipasena & Rha, 1977a, 1977b; Tang, Munro, & McCarthy, 1993). Modifications, such as heat induced aggregation, of whey proteins have been found to alter the functionality of the proteins (Bryant & McClements, 1998; Foegeding, Vardhanabhuti, & Yang, 2011; Hudson, Daubert, & Foegeding, 2000; Jeurnink & De Kruif, 1993; Resch & Daubert, 2002). Such modifications have been applied industrially to produce texturisers and thickeners for foods, and these have found application as fat replacers (Sandrou & Arvanitoyannis, 2000). Various fat replacers based on proteins are now available in the market (Prindiville, Marshall, & Heymann, 2000; Renard, Robert, Faucheron, & Sanchez, 1999; Sandrou & Arvanitoyannis, 2000). In a previous paper we reported on the structural characterization of partially denatured whey protein products (PDWPC's). We showed that it was possible to produce protein aggregates with differing structure by controlling the denaturation and aggregation process (Zhang, Arrighi, Campbell, Lonchamp, & Euston, 2016). PDWPC's can be formed with structures that are similar to the known gel structures formed by WPC solutions. That is PDWPC's with compact, densely packed structures that resemble particulate gels, with elongated tubular aggregates that resemble fibrilar gels, or with mixed structures can be formed depending on the processing conditions used. We would expect different functionalities to be obtained from such products due to their differing structures, and thus, studies on the rheological behaviour and deduction of the structure–functionality relationship of different PDWPC's are of importance in understanding these.

In this paper we study the concentration dependent flow

* Corresponding author.
E-mail address: S.R.Euston@hw.ac.uk (S.R. Euston).

http://dx.doi.org/10.1016/j.foodhyd.2015.12.012

behaviour, including shear thinning, and thixotropy properties of PDWPC's and from this the interactions of the modified protein molecules that control the solution behaviour are deduced. The flow behaviour of Simplesse, a microparticulated WPC (MPWPC) and non-denatured WPC are also studied for comparison purposes. In a future paper we will discuss the viscoelastic properties of solutions of the same protein products. The aim of this work is to understand the relationship between structure and rheological properties of PDWPC's and to use this information to inform the manufacture of PDWPC's with controlled thickening, and texture modifying properties.

2. Materials & methods

Whey protein concentrate (Lacprodan 87), was a gift from Arla Foods Ingredients, Denmark, Simplesse® 100 [E] a gift from CP Kelco UK Limited, and a series of partially denatured whey protein products (PDWPC's) were a gift from Nandi Proteins, Edinburgh, UK. The composition of the proteins according to the manufacturers is given in Table 1. The protein products were dissolved in Milli-Q water at room temperature to make solutions with protein concentrations of 6%, 9%,12%, 14%, 16%, 18% and 21% (w/w). The solutions were stirred gently for at least 1 h to allow hydration of the proteins. Details of the manufacturing process for the PDWPC's have been given in our previous paper (Zhang et al., 2016). Briefly, the PDWPC's were made from a sweet whey stream, heated under controlled conditions to a given degree of denaturation. This is monitored by following the change in the free sulphydryl content of the protein as it is heated. It has been shown (Zhang et al., 2016) that the free sulphydryl content initially increases as the protein structure unfolds, and then decreases as inter-molecular disulphide bonds form. Processing of the PDWPC's is then possible so that the free sulphydryl content in the aggregates is increased, but inter-molecular disulphide bonds are not allowed to form giving aggregates of protein that are soluble. The aggregate size and morphology can be altered by controlling the degree of denaturation, pH, heating temperature and total solids of the heated whey stream. Four PDWPC products (coded PDWPC-A, PDWPC-B, PDWPC-C and PDWPC-D) were made with differing particle size and aggregate morphology as shown in Table 2. Three types of particle morphology were observed, a compact globular structure which has similarities to the particulate gels observed for whey proteins (PDWPC-A) (Clark, Kavanagh & Ross-Murphy, 2001); a fibrilar or tubular phase separated structure, similar to fibrilar gels (PDWPC-D) (Clark et al., 2001); and a mixed morphology with features of both particulate and fibrilar structures (PDWPC-B) (Foegeding, Bowland & Hardin, 1995) . The structure of one PDWPC (PDWPC-C) could not be determined as the aggregates were not stable under the conditions used to prepare them for scanning electron microscopy (Zhang et al., 2016) Further details of the manufacturing process and micrographs of the structures for the PDWPC's used in this study are given in Zhang et al. (2016).

Table 1
Composition of WPC, MPWPC and PDWPC powders.

	Composition (% w/w)		
	L87	PDWPC	MPWPC
Protein	87	60	53
Lactose	2	24	34
Fat	4.5	6	4
Ash	2.5	6.5	5
Moisture	4	3.5	4

2.1. Rheological measurements

All rheological measurements were performed using a Bohlin Gemini stress-controlled rheometer (Malvern Instruments, UK), with 4°/40 mm cone and plate (gap 150 μm) at a temperature of 20 °C. Steady shear viscosity of solutions was determined by applying a steady shear rate in the range 10^{-3} to 100 s^{-1} for 5 min. The average shear viscosity was calculated in the region where a constant, steady-state viscosity was obtained. Thixotropy properties were measured through shear-rate sweep tests, where a range of shear rates from ~10^{-3} s^{-1} to ~100 s^{-1} were employed in an up-down mode, with a total test time of 1 h (30 min up sweep, 30 min down sweep). The area between the ascending and descending curves was calculated with the Bohlin software (Malvern Instruments, UK) and this was reported as the thixotropy. Step shear rate tests were performed on some samples by holding the shear rate at 1 s^{-1} for 1400 s, then increasing the shear rate instantaneously to 100 s^{-1} for 1400 s, and then lowering it again, instantaneously, back to 1 s^{-1} for a further 1400 s.

2.2. Molecular orientation and Péclet number

Proteins, even those with globular structures, cannot be considered as perfect spheres due to the molecular asymmetry. It is a common experience that the long axis of a particle tends to be aligned in the flow direction of the streamline to reduce the resistance. Therefore, molecular orientations of the proteins significantly affect the viscosity of the solutions. Orientation of protein molecules is determined by the hydrodynamic forces on proteins from solvents and the Brownian motions of the proteins themselves. Of these two factors hydrodynamic forces will favour alignment of the protein molecules with the solvent flow, whilst Brownian motion favours the random orientation of the proteins (Macosko, 1994; Willenbacher & Georgieva, 2013).

In order to evaluate the balance between hydrodynamic forces and Brownian motion, the Péclet number, *Pe*, is introduced, which compares the time scales of hydrodynamic (convective) and Brownian motions (Goodwin & Hughes, 2008; Macosko, 1994; Willenbacher & Georgieva, 2013). According to the Stokes–Einstein equation, the diffusion coefficient, *D*, for a particle with a radius of *r* is calculated as,

$$D = \frac{k_B T}{6\pi\eta r} \tag{1}$$

where k_B is the Boltzmann constant, *T* the absolute temperature (in K), and η the viscosity of the solution. It should be noted that the viscosity involved in calculating the diffusion coefficient is that for the solution (i.e. solvent plus particles), not the solvent alone, since the Péclet number depends on the diffusivity of an isolated particle in the system, which is also affected by neighbouring particles other than the solvent (Goodwin & Hughes, 2008; Macosko, 1994; Willenbacher & Georgieva, 2013). When calculating *Pe*, the characteristic distance for Brownian motion is the particle radius, *r*, while the characteristic time for flow is defined as the reciprocal of the shear rate, γ (Goodwin & Hughes, 2008). Therefore, the characteristic times, $t_{Brownian}$ for Brownian motions and *t*, for the flows are given as

$$t_{Brownian} = \frac{r^2}{6D} = \frac{6\pi\eta r^3}{k_B T} \tag{2}$$

and

Table 2
Particle characteristics and morphology of PDWPC products.

PDWPC	Particle size (μm)	Degree of denaturation (%)	Particle morphology
PDWPC-A	5.48	65	Compact, globular
PDWPC-B	3.0	45	Mixed
PDWPC-C	17.0	51	Could not be determined
PDWPC-D	17.0	93	Fibrillar/tubular

$$t = \frac{1}{\gamma} \tag{3}$$

Accordingly, the Péclet number, *Pe*, is expressed as

$$Pe = \frac{t_{Brownian}}{t} = \frac{6\pi r^3 \sigma}{k_B T} \tag{4}$$

where $\sigma = \eta\gamma$ is the shear stress, and values for r are taken from our previous paper (Zhang et al., 2016).

Using equation (4), time scales for Brownian-motion-induced randomization of the molecular orientations and flow-induced molecular alignments can be compared and the more significant effect deduced. For Pe << 1 Brownian randomization of orientations dominates over shear-induced for the proteins molecules, and thus, effects of molecular orientations on viscosity of protein solutions are negligible (Macosko, 1994; Willenbacher & Georgieva, 2013). Similarly, for Pe >> 1 the viscosity of a protein solution is dominated by shear-induced orientation effects.

3. Results and discussion

3.1. Shear rate dependence

The effects of shear rate on viscosity of different protein samples are shown in Figs. 1 to 6. For reference, shear rates approximately corresponding to a Pe = 10 are shown. We have chosen Pe = 10 to indicate where shear-induced effects will be much greater (10 times) than Brownian effects. It is found that all the samples exhibit shear-thinning behaviour. According to Tung (1978), shear thinning behaviour of protein dispersions results from alignment of the polypeptide chains under shear flows, during which the interactions, such as hydrogen bonds and electrostatic interactions, between the randomly oriented molecules are disrupted and new orientations of the protein molecules along shear planes with lower resistance to flows are established.

3.1.1. WPC solution

For the solutions of WPC *Pe* values are small (<< 1) at low shear rates, suggesting that the shear thinning behaviour of the WPC solutions results not from intermolecular interactions but to the change in orientation of proteins alone (Foss & Brady, 2000; Goodwin & Hughes, 2008; Macosko, 1994). In Fig. 1, solutions of WPC are found to have low viscosity and no shear thinning or thickening behaviour at large *Pe* values (>10), indicating weak interactions between the protein molecules, which are of the same magnitude as the effects of Brownian motions on the random orientations of the polypeptide chains. Above a shear rate of about 10 s^{-1} the viscosity for WPC solutions is independent of shear rate.

3.1.2. MPWPC solutions

Shear dependence of viscosity for MPWPC solutions is shown in Fig. 2. The *Pe* values for MPWPC solutions are observed to be larger than those of WPC, since the MPWPC has larger particle size and higher viscosity (Goodwin & Hughes, 2008; Macosko, 1994). Shear thinning behaviour was observed in all MPWPC solutions with large *Pe* (>10), indicating it is inter-particle interactions that determine the flow behaviour of the protein aggregates rather than

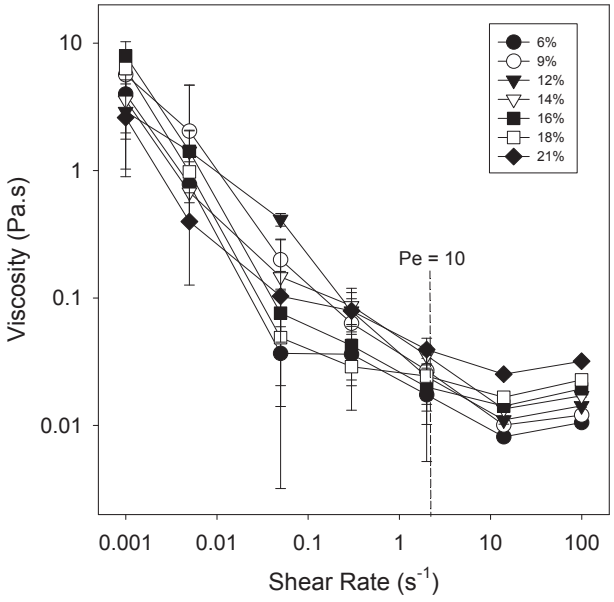

Fig. 1. Shear rate dependence of viscosity of WPC with 6%, 9%, 12% (■), 14%, 16%, 18%, and 21% protein concentrations (w/w). Error bars are ± one standard deviation of the mean.

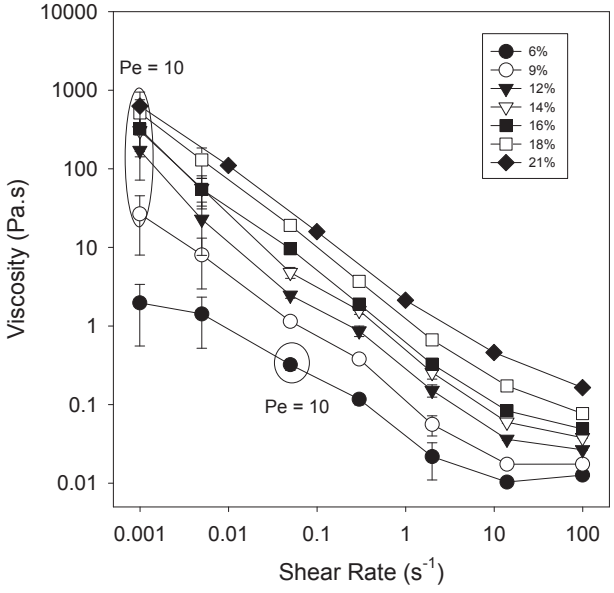

Fig. 2. Shear rate dependence of viscosity of MPWPC with 6%, 9%, 12%, 14%, 16%, 18%, and 21% protein concentrations (w/w). Error bars are ± one standard deviation of the mean.

Brownian motions (Barnes, Hutton, & Walters, 1989; Rao, 2007; Tung, 1978). At high shear rate (100 s^{-1}) in solutions with MPWPC concentrations of 9% (w/w) or less, a constant viscosity is reached suggesting that the inter-particle interactions between the MPWPC particles were completely disrupted by high shear stress (Barnes et al., 1989; Rao, 2007; Tung, 1978). According to Renard et al. (1999), such inter-particle interactions occur by flocculation of the MPWPC particles which form in the solution at rest and at low shear rates. In our previous paper (Zhang et al., 2016) MPWPC flocs were detected using particle size analysis and observed in scanning electron micrographs. Presumably, the large flocs are disrupted into small MPWPC particles at high shear rates, and thus give constant viscosity. This constant viscosity at high shear rates in MPWPC solutions disappears as the concentration of protein increases (12% (w/w), and above, Fig. 2).

3.1.3. PDWPC solutions

Flow behaviour of solutions of PDWPC proteins with a relatively small particle size (PDWPC-A and PDWPC-B) are shown in Figs. 3 and 4. The *Pe* values of PDWPC-A and PDWPC-B solutions were larger than those of MPWPC which is mainly due to the larger particle size and Pe > 10 was found at all shear rates and for all protein concentrations. Shear thinning properties with the absence of Newtonian plateaus at high shear rates were observed for all protein concentrations for PDWPC-A and PDWPC-B respectively as shown in Figs. 3 and 4, indicating that strong aggregated structures form at such protein concentrations.

The *Pe* values are large (>10,000) for all the solutions of PDWPC-C and PDWPC-D due to their large particle size (Zhang et al., 2016). Shear thinning behaviour at low shear rates and Newtonian plateaus at high shear rates were observed at lower protein concentrations (<12%) of these modified proteins as shown in Figs. 5 and 6. It is also found that shear thinning behaviour ceased at relatively low shear rates (~1 s^{-1}), indicating that there are no flocs formed in such dilute solutions, suggesting the aggregates align along the shear planes around the shear rate ~1 s^{-1} and above. Large increases in viscosity, especially at high shear rates, were observed in the solutions with protein concentrations of 12% and above for both PDWPC-C and PDWPC-D. The Newtonian plateaus observed at high shear rates for the lower protein concentration solutions disappear in the solutions of 12% protein concentration and above. As shown in Figs. 1 to 6, modified proteins and WPC have similar viscous behaviour in their dilute solutions at high shear rates, indicating the protein molecules or particles have similar hydrodynamic interaction when their inter-particle interactions are not significant and when they are completely aligned along the shear planes (Matveenko & Kirsanov, 2011; Tung, 1978). In concentrated solutions, however, modified proteins particles exhibit higher resistance to flows than WPC even at high shear rates, indicating strong inter-particle interactions between the former. Newtonian plateaus

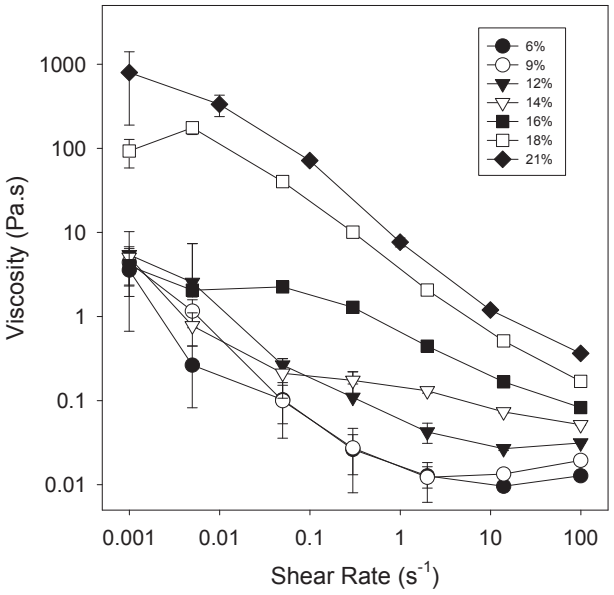

Fig. 4. Shear rate dependence of viscosity of PDWPC-B with 6%, 9%, 12%, 14%, 16%, 18%, and 21% protein concentrations (w/w). Error bars are ± one standard deviation of the mean.

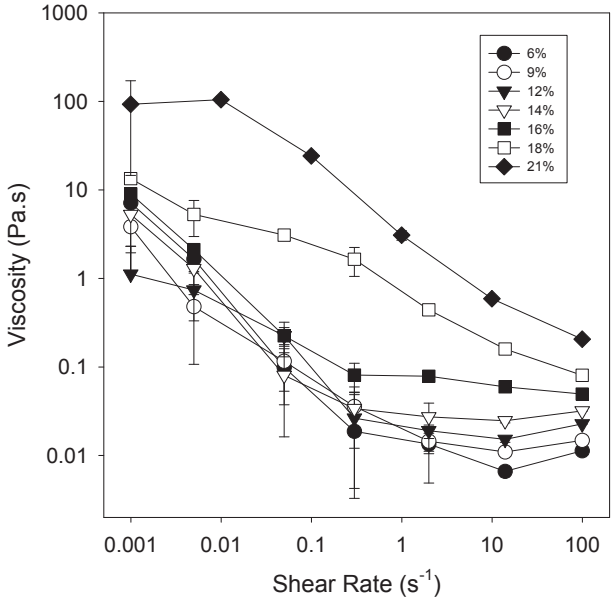

Fig. 3. Shear rate dependence of viscosity of PDWPC-A with 6%, 9%, 12% (■), 14%, 16%, 18%, and 21% protein concentrations (w/w). Error bars are ± one standard deviation of the mean.

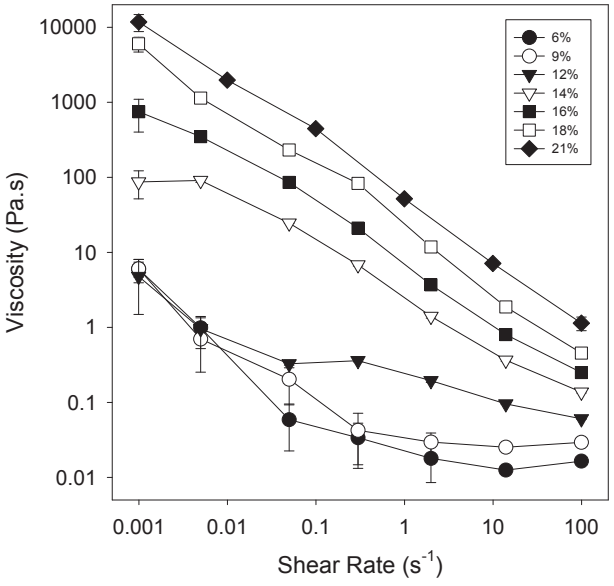

Fig. 5. Shear rate dependence of viscosity of PDWPC-C with 6%, 9%, 12%, 14%, 16%, 18%, and 21% protein concentrations (w/w). Error bars are ± one standard deviation of the mean.

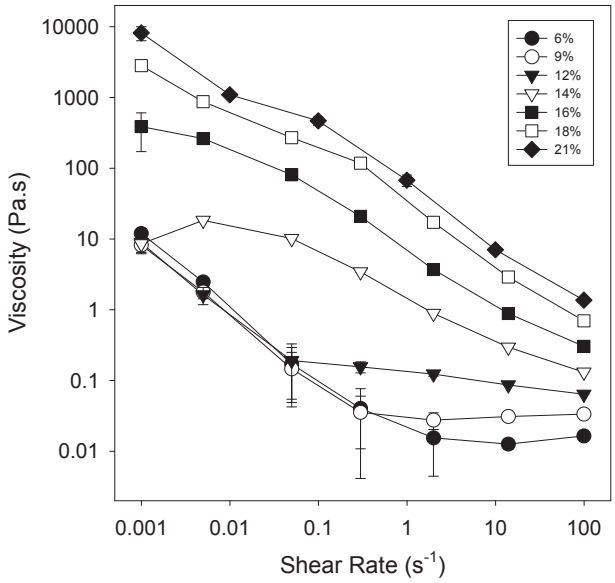

Fig. 6. Shear rate dependence of viscosity of PDWPC-D with 6%, 9%, 12%, 14%, 16%, 18%, and 21% protein concentrations (w/w). Error bars are ± one standard deviation of the mean.

at high shear rates are absent in concentrated solutions of modified proteins, suggesting the interactions between the flowing aggregates prevent them from achieving complete alignment.

3.2. Thixotropy

Shear sweeps were carried out for solutions of WPC, MPWPC and PDWPC's at various concentrations by increasing the shear rate to 100s^{-1} and then decreasing it. The deviation of the two curves from each other reveals thixotropy properties, is termed hysteresis or thixotropy and indicates a time delay in the reforming of interactions that are broken by high shear (Green & Weltmann, 1943; Mewis, 1979; Mewis & Wagner, 2009). Shear sweep plots of shear stress against shear rate are given as supplementary material in Figs. S1–S6 and the thixotropy is summarised in Fig. 7. It is found that all modified proteins, (MPWPC and PDWPC's), display thixotropic behaviour in concentrated solutions, but that the WPC solutions do not. The ascending shear stresses were observed to be larger than descending ones for all the thixotropic samples, indicating that aggregates formed between the protein particles at rest or low shear rate in concentrated solutions are disrupted by strong shear flows, and that disrupted structures do not reform immediately (Tung, 1978). The hysteresis phenomenon results from the retarded Brownian motions caused by large viscosity, and therefore, it can take a long time for the aligned flowing units, such as protein molecules or protein aggregates, to recover their random orientations or reform the aggregation structures (Mewis, 1979; Tung, 1978). It should be noted that the observation of thixotropic properties of MPWPC disagrees with the observations of Renard et al. (1999), who found that MPWPC exhibit anti-thixotropic behaviour where increased shear promotes ordered structure formation. This difference could be because Renard et al. (1999) used increased ionic strength in their MPWPC solutions (they were suspended in 0.1 M NaCl) whilst our measurements were carried out in the absence of NaCl. Since electrostatic repulsions are shielded by ions in the solution (Bryant & McClements, 1998; Renard et al., 1999), it is much easier for the MPWPC to approach each other and flocculate again when salt is present.

The surface area of the hysteresis loops, which represents the

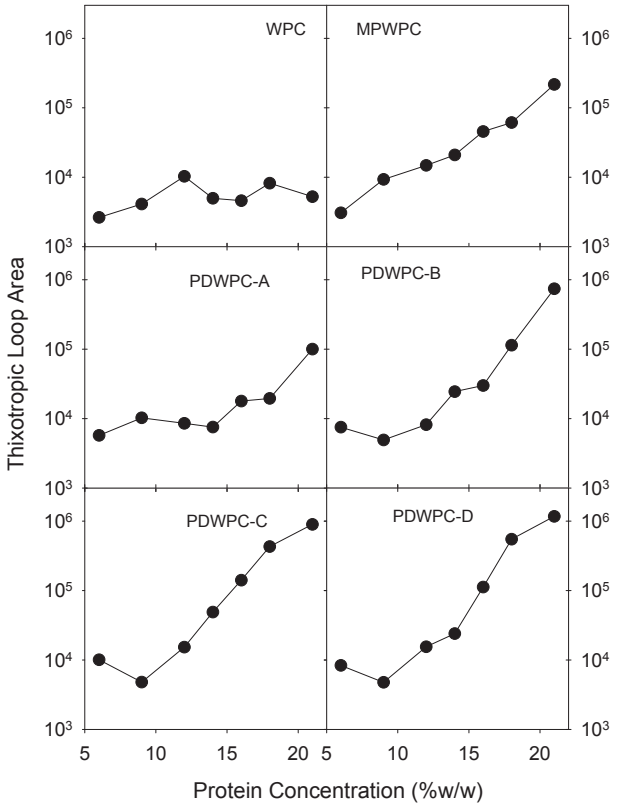

Fig. 7. Difference in the thixotropic loop area (TLA) between increasing and decreasing shear rate curves for the WPC, MPWPC and PDWPC products.

difference in the rate of energy dissipation between ascending and descending flows (Kirsanov, Remizov, Novoselova, & Matveenko, 2007; Matveenko & Kirsanov, 2011), is often used to evaluate the degree of thixotropy (Green & Weltmann, 1943; Mewis, 1979; Mewis & Wagner, 2009). The thixotropic loop area between the two flow curves, which we have denoted as TLA, as calculated by the rheometer software is plotted in Fig. 7. Only small changes in TLA were observed for solutions of WPC at different concentrations which are within the experimental error range, indicating the absence of thixotropy from these samples. Similar behaviour is observed in the dilute solutions of MPWPC and PDWPC proteins at concentrations below about 12% (w/w). However, large increases in TLA were found in concentrated solutions of the modified proteins, where structure is formed between aggregates as concentration increases (Tung, 1978).

The critical concentrations of the protein solutions at which *TLA* start to increase rapidly are listed in Table 3. It is found that in solutions of PDWPC's with large aggregates (PDWPC-C and PDWPC-D) the particles interact with each other at lower concentrations, while MPWPC and products with small aggregates (PDWPC-A and PDWPC-B) need higher concentrations for inter-

Table 3
Critical concentrations for large increases thixotropic loop area (TLA).

	Protein concentration (%, w/w)	Increase in *TLA* ($\times 10^4$)
WPC	–	–
MPWPC	14–16	2.47
PDWPC-A	14–16	1.03
PDWPC-B	12–14	1.62
PDWPC-C	9–12	1.05
PDWPC-D	9–12	1.07

particle interaction. In our previous paper (Zhang et al., 2016) it was shown that PDWPC-D, in particular, had very open particles made up of interconnecting tubules, compared to much more compact particles for PDWPC-A, PDWPC-B and MPWPC. Larger more open particles will occupy more space in solution for a given weight concentration which explains why they interact more strongly at lower concentrations than do the smaller more compact particles.

It is not clear from shear sweep data what the origin of the thixotropic loop is. To better understand where the difference in viscosity between the increasing and decreasing shear rate components of the shear sweep comes from, we carried out stepped shear rate tests on solutions of all protein samples containing 21% protein (w/w). In this the sample has been sheared at 1 s^{-1} for 1400 s, which is then increased instantaneously to 100 s^{-1} for, and then decreased instantaneously to 1 s^{-1} for a further 1400 s. The results for this are show in Fig. 8. The results show clearly that for the WPC the viscosity is independent of shear rate between 1 s^{-1} and 100 s^{-1}. For MPWPC, the solution is strongly shear thinning, and after shearing at high shear rate and a decrease in shear rate to 1 s^{-1} the viscosity shows a delayed return to the viscosity seen in the first shearing period at 1 s^{-1}. This indicates thixotropy and that the structure of the individual particles is unaltered by shear. For the PDWPC particles two types of behaviour are observed. For PDWPC-A and PDWPC-B, the solutions are shear thinning, and exhibit some thixotropy (delayed recovery of the viscosity) but do not return to the viscosity observed for the first period of shearing at 1 s^{-1}. This suggests that at least part of the thixotropic loop observed in the shear sweeps can be explained by irreversible disruption of the particles by the high shear rate. For PDWPC-C and PDWPC-D, again shear thinning is observed when the shear rate is increased, but for these PDWPC's the increase in viscosity is virtually instantaneous when the shear rate is decreased back to 1 s^{-1}. The viscosity in the second period of shearing at 1 s^{-1} is lower than in the first, which again suggests irreversible changes to the structure of the particles at high shear rate. This would suggest that PDWPC's C and D are not thixotropic but the particles are sensitive to the high shear rate.

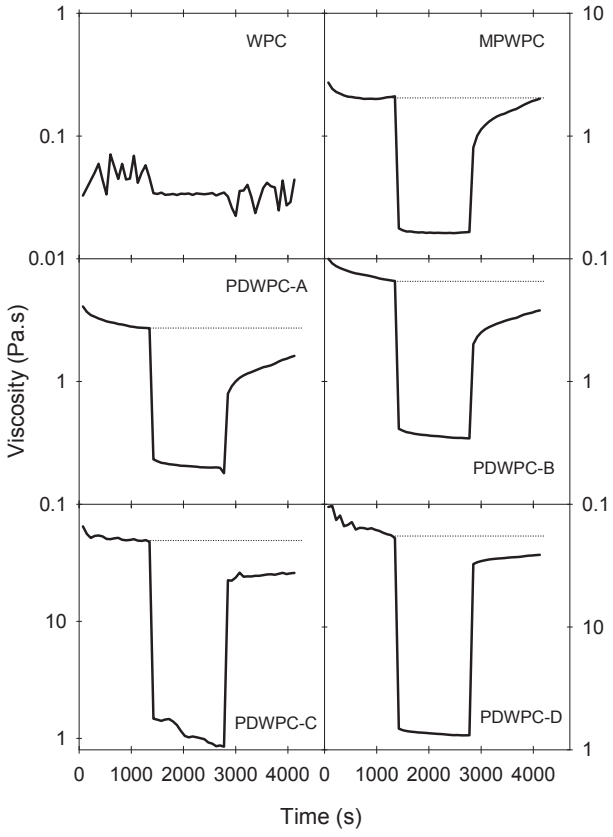

Fig. 8. Stepped shear rate tests for WPC, MPWPC and PDWPC samples at 21% protein (w/w). The sample is sheared at 1 s^{-1} for 1400 s, then at 100 s^{-1} for 1400 s, and then again at 1 s^{-1} for a further 1400 s. The dotted lines are included to indicate the final viscosity value during the initial 1 s^{-1} shearing period.

3.3. Concentration dependence of solution shear viscosity

3.3.1. Scaling relationship of viscosity to concentration

In solution interactions between protein and water molecules and interactions between more than one protein molecules gives rise to the viscosity of the protein solutions (Barnes et al., 1989; Damodaran, 1996; Rao, 2007). The protein–protein interactions depend mainly on the volume fraction (φ) occupied by the protein molecule (Macosko, 1994). Accordingly, dependence of flow behaviour on weight concentration (w) of a protein solution reveals the interactions between molecules in the system, based on the assumption of proportionality of volume fraction with weight fraction at moderate concentrations (Pradipasena & Rha, 1977a; Tang et al., 1993). This relationship between volume fraction and weight fraction was demonstrated for all of the protein products in our previous paper (Zhang et al., 2016).

We have analysed the viscosity vs concentration data using scaling relationships. Scaling theory was developed for and is perhaps more relevant to polymer solutions (De Gennes, 1979) but the general idea can be used to analyse our protein products. To analyse the concentration dependence of the protein products we will assume that $\eta \sim w^n$, where η is the viscosity, w is the protein concentration in wt%, and n is a characteristic coefficient for a particular solution. If this relationship holds, a plot of log (η) vs log

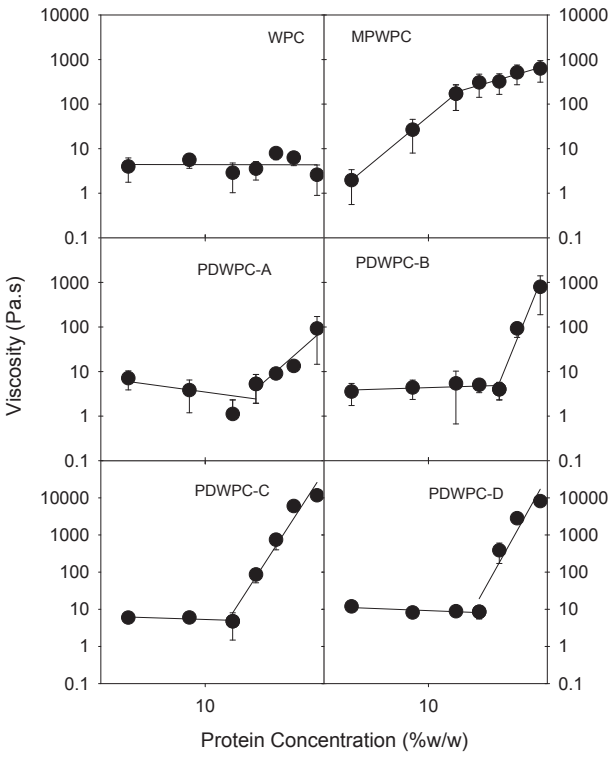

Fig. 9. Log–log plots of the concentration dependence of the apparent viscosity at 0.001 s^{-1}. Error bars are ± one standard deviation of the mean.

(w) will be linear with slope n. The value of the exponent n describes the rate of change of viscosity with concentration.

Fig. 9 shows this plot for the WPC, MPWPC and PDWPC solutions with η measured at a shear rate of 0.001 s^{-1} and Fig. 10 for a shear rate of 100s^{-1}. The relationship between viscosity and protein content is complex. At low shear rate (0.001 s^{-1}) the viscosity of WPC is found to be independent of protein concentration (Fig. 9). This is also true for PDWPC samples which show a concentration independent shear viscosity below critical concentrations of 12–16% (depending on sample). The exception to this is MPWPC where the shear viscosity at 0.001 s^{-1} is an increasing function of concentration over the whole range studied. Several researchers have reported that globular proteins form ordered, solid-like colloidal crystal phases at relatively low concentrations under conditions of zero or very low shear (Matsumoto & Inoue, 1996; Ikeda & Nishinari, 2000, 2001a, 2001b). These are formed because the proteins interact through weak long range repulsive electrostatic interactions. Although these electrostatic interactions are weak, they have a relaxation time that is much shorter than the characteristic time of flow, and therefore may appear to be undisrupted over the long time scale of the measurement. One observation that should be made, however, is that these protein colloidal crystals have been observed at concentrations much lower (<1% w/w) than used here and so the explanation for the concentration independence of the viscosity of WPC solutions seen here will be more complex, and may involve a combination of electrostatic interaction and volume exclusion effects at higher packing densities of the proteins. For the PDWPC particles, another form of interaction must become important above a critical concentration since the viscosity starts to increase. This is likely to be a frictional or hydrodynamic interaction between the surfaces of adjacent particles or between hydration layers around particles which only occur at short range and become important as the protein concentration increases and the particles get closer together. This interaction is obviously not as important in WPC solutions, possibly because the native protein molecules are less rigid and more deformable in solution.

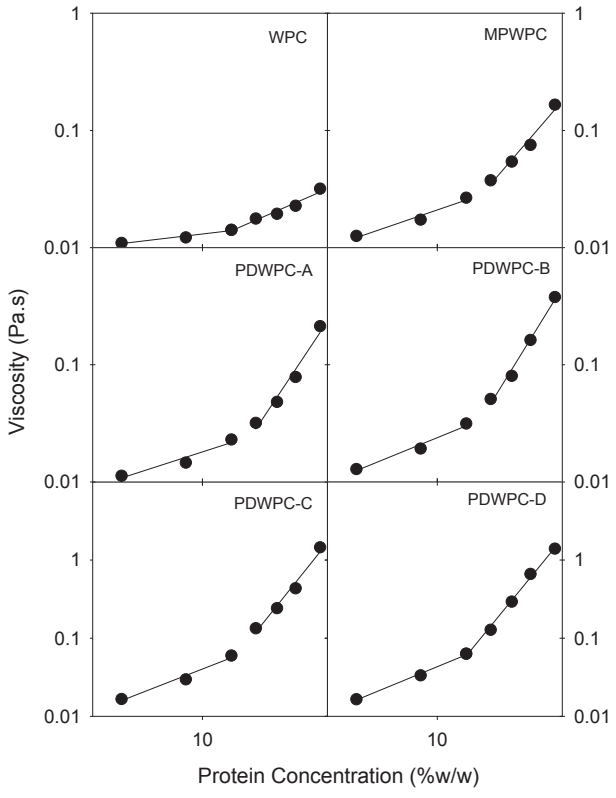

Fig. 10. Log–log plots of the concentration dependence of the apparent viscosity at 100 s^{-1} (error bars are too small to be visible on these plots).

The MPWPC solutions do not exhibit a region where viscosity is independent of concentration. Instead, the viscosity increases as concentration increases, but there is a change in slope in the log–log plots (Fig. 9) at a concentration of 12 wt% protein. At concentrations above the critical concentration the exponent n (rate of viscosity change) differs between the PDWPC products and MPWPC. MPWPC has the lowest value of n at 2.27 followed by PDWPC-A (n = 6.86). PDWPC-B to D have a much greater rate of change in viscosity (two-three times greater than for PDWPC-A). To explain this differing behaviour we should consider the way in which the protein products are manufactured and the microstructure of the aggregated particles. PDWPC's are heated at temperatures below the denaturation temperature, and unfolding of the protein will be limited. MPWPC on the other hand is heated extensively at temperatures above the denaturation temperature leading to extensive tertiary structure unfolding and aggregation. One might expect the MPWPC particles to be larger than those of the PDWCs. However, during MPWPC manufacture the solution is sheared to break up protein aggregates into smaller particles around a micron in size. The more extensive denaturation of proteins in MPWPC will lead to their having more exposed hydrophobic regions on their surface compared to the partially denatured PDWPC protein particles. This would lead to flocculation of the MPWPC, a phenomenon we have previously reported for MPWPC (Zhang et al., 2016). Thus in MPWPC we believe that hydrophobic interactions may be important at lower concentrations, and a combination of hydrophobic and hydrodynamic interactions at higher concentrations, with both of these dominating over any structuring due to weak long-range repulsive electrostatic forces.

The differences in viscosity concentration dependence at low shear rate for PDWPCs cannot be explained in the same way. Here, it is likely that differences in the microstructure of the particles contribute to the interactions between particles thus influencing viscosity. PDWPC-B has the highest rate of change of viscosity with concentration above the critical concentration (n = 19.18) compared to n = 14.46 and n = 16.74 for PDWPC-C and PDWPC-D respectively. Compared to PDWPC-A the aggregates in PDWPC-B were slightly smaller, but had a more open and porous structure, whilst PDWPC-D (which has considerably larger particles than PDWPC-B) had a more fibril-like structure (Zhang et al., 2016). In this previous study we proposed that PDWPC-B structure was closer to that of a mixed gel, whilst PDWPC-A was particulate-like and PDWPC-D more like a fibrillar gel. Presumably, the PDWPC-B structure has a greater interaction than the other PDWPCs at low shear rate, possibly because the surface of the particles is rougher than for the other PDWPC's. The effect of surface roughness on the viscosity of suspensions of particles is complex and difficult to study experimentally in a systematic way. Computer simulations have been used to understand some of the general features of the viscosity of rough particles (Wilson & Davis, 2000; 2002). The simulations suggest that at low particle concentrations surface roughness actually decreases viscosity, because the surface imperfections reduce close approach of particles thus reducing interactions (Wilson & Davis, 2000). At higher particle densities, however, where particles are closer together surface roughness has the opposite effect and increases solution viscosity (Wilson & Davis, 2000).

The two distinct regions of concentration dependence in viscosity reveal a transition of the solution behaviour. This may be the similar to the crossover from a ‘dilute’ behaviour to a ‘semi-dilute’

behaviour which occurs in polymer solutions (Dickinson & Stainsby, 1982; Tang et al., 1993). This is often termed the crossover concentration and corresponds to the concentration at which polymers start to interact sterically through interpenetration and overlap. For the particles in this study, the crossover may indicate a change from a system where long-range electrostatic inter-particle interactions are important to one where short-range hydrodynamic interactions become more important.

At high shear rate (100 s^{-1}) the Pe number is high for all protein samples at all concentrations and shear effects will dominate. When viscosity at 100 s^{-1} is plotted vs concentration on a log–log plot (Fig. 10), the regions where viscosity is independent of concentration disappear, and there are found to be two linear regions with differing concentration dependence for all protein product solutions. At high shear rate any weak repulsive electrostatic interactions that order the proteins are disrupted, and from the stepped shear tests we also know that for the PDWPC samples some irreversible changes to the solutions occurs. For WPC solutions two linear regions are seen in Fig. 10 for protein concentrations <12% (w/w) and for concentrations >12% (w/w). These linear regions indicate a power law scaling of viscosity with protein concentration (w) i.e. $\eta \sim w^n$. A power law relationship reveals the existence of protein–protein interactions, which increase as the number of protein molecules increases, with a concomitant increase in the viscosity of the solution (Lizarraga et al., 2006; Macosko, 1994; Pradipasena & Rha, 1977b; Tang et al., 1993). Similarly, two concentration regimes were also observed for both MPWPC and PDWPC proteins, again suggesting a crossover between two forms of particle interaction.

The boundary of the concentration regimes and concentration dependence of viscosity (n), for each sample are listed in Table 4. As the concentration increases, the concentration dependences of viscosity of modified proteins are much larger than that of the WPC, indicating that there are stronger intermolecular interactions existing between those modified protein molecules than for WPC, and these forces facilitate the structure formation in the modified proteins (Elofsson, Dejmek, Paulsson, & Burling, 1997; Ju & Kilara, 1998). The rate of change of viscosity at 100 s^{-1} in regime 2 is substantially lower than for the same regime at a shear rate of 0.001 s^{-1}. The rate of viscosity change with concentration for MPWPC solutions in regime 1 (Table 4) is similar to those of the partially denatured proteins with small aggregates (i.e., PDWPC-A and PDWPC-B) and WPC, but smaller than those of the partially denatured proteins with large aggregates (i.e., PDWPC-C and PDWPC-D). This suggests that microparticulated proteins and partially denatured protein products with a low degree of aggregation have similar interactions between molecules with the absence of structure formation at high shear rate, while the highly aggregated partially denatured proteins (PDWPC-C and PDWPC-D) exhibit stronger resistance to flows, perhaps due to the elongated tubular nature of their particle structure.

In Regime 2 (Table 4), MPWPC is found to possess a higher concentration dependence of viscosity than at lower concentrations, but this is smaller than for the partially denatured protein aggregates. Clearly, at the crossover concentration there is a change in the way in which particles interact with each other and this is different between the protein products. In our previous paper (Zhang et al., 2016) based on SEM micrographs we observed a tendency for MPWPC particles to form flocs. These flocs increase the resistance to flow at high concentrations through the interactions between hydration shells of the constituent particles (Ikeda & Nishinari, 2000, 2001; Renard et al., 1999). A floc of particles will behave as a single particle with a volume and diameter that is bigger than the sum of the constituent particles. This is because the flocs entrap water in spaces inside the structure and increase the effective volume fraction of the particles.

Krieger (1972) found that there is no dependence of viscosity on particle size at a constant particle volume fraction. We see this in our results where PDWPC-A has a larger particle size than PDWPC-B but a lower viscosity (Figs. 9 and 10) and PDWPC-C and PDWPC-D have the same particle size but differing viscosity (Figs. 9 and 10). This suggests that differences in viscosity between the protein products arise from differences in the morphology and structure of the particles and the way that the particles interact with each other or with water. From our previous studies we know that MPWPC and PDWPC-A particles have a similar structure, albeit on a different scale (Zhang et al., 2016). Scanning electron micrographs of particles of these products showed a structure that is compact and cauliflower-like in appearance (Zhang et al., 2016). PDWPC-B on the other hand, has a more open structure and a rougher particle surface (Zhang et al., 2016). Given these differences it is not unreasonable to suggest that the hydration properties of the particles (i.e. how they interact with water) will differ significantly between the two types of particle structure, and this will lead to differences in solution viscosity, even though they have similar particle size. PDWPC-D in particular has a highly elongated tubule-like structure (Zhang et al., 2016) with the tubules joined together to form a porous particle, which is much different to the particulate structures of MPWPC, PDWPC-A and PDWPC-B.

4. Conclusions

The flow properties of partially denatured whey protein aggregates are complex and depend on the micro-structural

Table 4
Crossover concentrations and concentration dependence of viscosity (n) of for WPC, MWPC and PDWPC samples.

	Particle size D (0.5) (μm)	Shear rate (s^{-1})	Crossover concentration (%, w/w)	Concentration dependence (n)	
				Regime 1	Regime 2
WPC	0.48 ± 0.04	0.001	–	–	–
		100	12	0.38	1.37
MPWPC	1.72 ± 0.04	0.001	12	6.44	2.27
		100	12–14	1.06	3.62
PDWPC-A	5.48 ± 0.001	0.001	14	–	6.86
		100	12–14	1.00	4.68
PDWPC-B	3.30 ± 0.001	0.001	16	–	19.18
		100	12–14	1.27	5.04
PDWPC-C	17.0 ± 0.70	0.001	12	–	14.46
		100	12–14	1.82	5.85
PDWPC-D	17.0 ± 1.00	0.001	14	–	16.74
		100	12	1.93	5.70

morphology of the particles, the particle concentration as well as the shear rate. The shear viscosity behaviour of PDWPC's differs markedly from that of both WPC and MPWPC. Much of these differences, we believe, can be explained by the morphological differences in the structure of aggregated protein particles in MPWPC and PDWPC. High viscosity is found for particles with open, fibril/tubule-like structures, whilst the compact aggregates have lower viscosity. The results from this study suggest that there is scope to use partial denaturation technology to control structure of WPC aggregates to target specific viscosity characteristics. However, to achieve this will require a more detailed knowledge of how processing factors can be related to PDWPC aggregate structure and functional properties. This work is ongoing and will include studies on the rheological properties of controlled aggregates structures, as well as additional research on PDWPC emulsifying and foaming properties.

Acknowledgements

SRE acknowledges the Heriot-Watt University Life Science-Physical Science Interface Theme for the award of a PhD Scholarship to Zhuo Zhang and the Engineering & Physical Sciences Research Council for grant No. EP/J501682/1.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.foodhyd.2015.12.012.

References

Barnes, H. A., Hutton, J. F., & Walters, K. (1989). *An introduction to rheology*. Amsterdam, the Netherlands: Elsevier.
Bryant, C. M., & McClements, D. J. (1998). Molecular basis of protein functionality with special consideration of cold-set gels derived from heat-denatured whey. *Trends in Food Science & Technology, 9*(4), 143–151.
Clark, A. H., Kavanagh, G. M., & Ross-Murphy, S. B. (2001). Globular protein gelation – theory and experiment. *Food Hydrocolloids, 15*, 383–400.
Damodaran, S. (1996). Amino acids, peptides, and proteins. In O. R. Fennema (Ed.), *Food chemistry* (3rd ed.). New York, US: Marcel Dekker, Inc.
De Gennes, P. G. (1979). *Scaling concepts in polymer physics*. Ithaca, USA: Cornell University Press.
Dickinson, E., & Stainsby, G. (1982). *Colloids in food*. London, United Kingdom: Applied Science.
Elofsson, C., Dejmek, P., Paulsson, M., & Burling, H. (1997). Characterization of a cold-gelling whey protein concentrate. *International Dairy Journal, 7*(8–9), 601–608.
Foegeding, E. A., Bowland, E. L., & Hardin, C. C. (1995). Factors that determine the fracture properties and microstructure of globular protein gels. *Food Hydrocolloids, 9*, 237–249.
Foegeding, E. A., Vardhanabhuti, B., & Yang, X. (2011). Dairy systems. In I. T. Norton, F. Spyropoulos, & P. Cox (Eds.), *Practical food rheology: An interpretive approach*. Wiley-Blackwell.
Foss, D. R., & Brady, J. F. (2000). Structure, diffusion and rheology of Brownian suspensions by Stokesian dynamics simulation. *Journal of Fluid Mechanics, 407*, 167–200.
Goodwin, J. W., & Hughes, R. W. (2008). *Rheology for chemists-an introduction* (2nd ed.). Cambridge, UK: The Royal Society of Chemistry.
Green, H., & Weltmann, R. N. (1943). Analysis of the thixotropy of pigment-vehicle suspensions basic principles of the hysteresis loop. *Industrial and Engineering Chemistry Analytical Edition, 15*(3), 201–206.
Harper, W. J. (2004). *Biological properties of whey components: A review: Update 2004*. Elmhurst, IL, US: American Dairy Products Institute.
Hermansson, A.-M. (1975). Functional properties of proteins for foods-flow properties. *Journal of Texture Studies, 5*(4), 425–439.
Hudson, H. M., Daubert, C. R., & Foegeding, E. A. (2000). Rheological and physical properties of derivitized whey protein isolate powders. *Journal of Agricultural and Food Chemistry, 48*(8), 3112–3119.
Ikeda, S., & Nishinari, K. (2000). Intermolecular forces in bovine serum albumin solutions exhibiting solid like mechanical behaviours. *Biomacromolecules, 7*(4), 757–763.
Ikeda, S., & Nishinari, K. (2001a). On solid-like rheological behaviors of globular protein solutions. *Food Hydrocolloids, 15*, 401–406.
Ikeda, S., & Nishinari, K. (2001b). Solid-like mechanical behaviours of ovalbumin aqueous solutions. *International Journal of Biological Macromolecules, 28*, 315–320.
Jeurnink, T. J. M., & De Kruif, K. G. (1993). Changes in milk on heating: viscosity measurements. *Journal of Dairy Research, 60*, 130–150.
Ju, Z. Y., & Kilara, A. (1998). Textural properties of cold-set gels induced from heat-denatured whey protein isolates. *Journal of Food Science, 63*(2), 288–292.
Kinsella, J. E., & Whitehead, D. M. (1988). Proteins in whey: chemical, physical, and functional properties. *Advances in Food and Nutrition Research, 33*, 343–438.
Kirsanov, E. A., Remizov, S. V., Novoselova, N. V., & Matveenko, V. N. (2007). Physical meaning of the rheological coefficients in the generalized casson model. *Moscow University Chemistry Bulletin, 62*(1), 18–21.
Krieger, I. M. (1972). Rheology of monodisperse latices. *Advance in Colloid Interface Science, 3*, 111–136.
Lizarraga, M. S., De Piante Vicin, D., González, R., Rubiolo, A., & Santiago, L. G. (2006). Rheological behaviour of whey protein concentrate and λ-carrageenan aqueous mixtures. *Food Hydrocolloids, 20*(5), 740–748.
Macosko, C. W. (1994). *Rheology: Principles, measurements, and applications*. New York, USA: Wiley-VCH, Inc.
Madureira, A. R., Pereira, C. I., Gomes, A. M. P., Pintado, M. E., & Xavier Malcata, F. (2007). Bovine whey proteins – overview on their main biological properties. *Food Research International, 40*(10), 1197–1211.
Matsumoto, T., & Inoue, H. (1996). Colloidal structure and properties of bovine serum globulin aqueous systems using SAXS and rheological measurements. *Chemical Physics, 207*, 167–172.
Matveenko, V. N., & Kirsanov, E. A. (2011). The viscosity and structure of dispersed systems. *Moscow University Chemistry Bulletin, 66*(4), 199–228.
McDonough, F. E., Hargrove, R. E., Mattingly, W. A., Posati, L. P., & Alford, J. A. (1974). Composition and properties of whey protein concentrates from ultrafiltration. *Journal of Dairy Science, 57*(12), 1438–1443.
Mewis, J. (1979). Thixotropy – a general review. *Journal of Non-Newtonian Fluid Mechanics, 6*(1), 1–20.
Mewis, J., & Wagner, N. J. (2009). Thixotropy. *Advances in Colloid and Interface Science, 147–148*, 214–227.
Pradipasena, P., & Rha, C. (1977a). Effect of concentration on apparent viscosity of a globular protein solution. *Polymer Engineering and Science, 17*(12), 861–864.
Pradipasena, P., & Rha, C. (1977b). Pseudoplastic and rheopectic properties of a globular protein (β-lactoglobulin) solution. *Journal of Texture Studies, 8*(3), 311–325.
Prindiville, E. A., Marshall, R. T., & Heymann, H. (2000). Effect of milk fat, cocoa butter, and whey protein fat replacers on the sensory properties of lowfat and nonfat chocolate ice cream. *Journal of Dairy Science, 83*(10), 2216–2223.
Rao, M. A. (2007). *Rheology of fluid and semisolid foods principles and applications*. New York, USA: Springer Science+Business Media.
Renard, D., Robert, P., Faucheron, S., & Sanchez, C. (1999). Rheological properties of mixed gels made of microparticulated whey proteins and b-lactoglobulin. *Colloids and Surfaces B: Biointerfaces, 12*, 113–121.
Resch, J. J., & Daubert, C. R. (2002). Rheological and physicochemical properties of derivatized whey protein concentrate powders. *International Journal of Food Properties, 5*(2), 419–434.
Sandrou, D. K., & Arvanitoyannis, I. S. (2000). Low-fat/calorie foods: current state and perspectives. *Critical Reviews in Food Science and Nutrition, 40*(5), 427–447.
Séverina, S., & Xia, W. (2005). Milk biologically active components as nutraceuticals: review. *Critical Reviews in Food Science and Nutrition, 45*(7–8), 645–656.
Tang, Q., Munro, P. A., & McCarthy, O. J. (1993). Rheology of whey protein concentrate solutions as a function of concentration, temperature, pH and salt concentration. *Journal of Dairy Research, 60*, 349–361.
Tung, M. A. (1978). Rheology of protein dispersions. *Journal of Texture Studies, 9*(1–2), 3–31.
Willenbacher, N., & Georgieva, K. (2013). Rheology of disperse systems. In U. Böckel, W. Meir, & G. Wagner (Eds.), *Product design and engineering: Formulation of gels and pastes*. Weinheim, Germany: Wiley-VCH Verlag GmbH & Co.
Wilson, H., & Davis, R. (2000). The viscosity of a dilute suspension of rough spheres. *Journal of Fluid Mechanics, 421*, 339–367.
Wilson, H., & Davis, R. (2002). Shear stress of a monolayer of rough spheres. *Journal of Fluid Mechanics, 452*, 425–441.
Zhang, Z., Arrighi, V., Campbell, L., Lonchamp, J., & Euston, S. R. (2016). Properties of partially denatured whey protein products: formation and characterisation of structure. *Food Hydrocolloids, 52*, 95–105.